REMARKS

With this response, claims 1-3 and 5-21 have been cancelled. Claims 22-68 are newly added. Support for claims 22, 35, 48, 62, and 63 can be found in the specification at p. 1, lines 26-32; Example 1, p. 13, lines 6-26; and original claims 1 and 11. Support for claims 23, 24, 36, 37, 49, 50, 64, and 65 can be found in the specification at p. 1, lines 10-14 and in Figure 1. Support for claims 25, 26, 27, 38, 39, 40, 51, 52, 53, 66, and 67 can be found in the specification in Example 1, p. p. 13, lines 16-25. Support for claims 28, 41, 54, and 68 can be found in original claim 3. Support for claims 29, 42, and 55 can be found in original claim 4. Support for claims 30, 43, and 56 can be found on original claim 12. Support for claims 31, 33, 44, 46, 57, and 59 can be found in the specification in Example 4, p. 19, line 1 through p. 20, line 6; Table 6, pp. 19-20; and Table 7, p. 20. Support for claims 32, 45, and 58 can be found in the specification in Table 1, p. 16. Support for claims 34, 47, and 60 can be found in the specification in Example 5, p. 21, line 1 through p. 22, line 16. Support for claims 61 can be found in the specification at p. 6, lines 24-25. Attached hereto is a marked-up version of the changes made by this amendment. The attached page is captioned "Version With Markings to Show Changes Made."

I. 35 U.S.C. 102(e) Rejection

Applicants acknowledge the Office's reconsideration and withdrawal of the rejection of claims 1-3, 5-8, and 10-14 as being unpatentable over Blumberg et al. (U.S. Patent No. 5,763,215) or Pedersen et al. (U.S. Patent No. 5,783,413).

II. 35 U.S.C. 103(a) Rejection

Reconsideration is requested of the rejection of claims 1-3 and 5-14 under 35 U.S.C. 103(a) as being unpatentable over Blumberg et al. (U.S. Patent No. 5,763,215) and Pedersen et al. (U.S. Patent No. 5,783,413) in view of Harper et al. (U.S. Patent No. 4,900,673) and Obata et al. (JP 07289256) or Obata et al. (1997).

Claims 1-3 and 5-14 have been canceled, thus rendering the rejection moot as applied thereto. These claims, however, have been replaced by claims 22-60 which are patentable over the cited references for the following reasons.

Claim 22 is directed to a method of removing an alanyl residue from the N-terminal region of a polypeptide. The method comprises expressing a peptide having an alanyl residue in the N-terminal region and contacting the expressed polypeptide with immobilized *Aeromonas proteolytica* aminopeptidase to cleave the alanyl residue.

Blumberg et al. disclose what they generally characterize as a method of removing N-terminal amino residues from eucaryotic polypeptide analogs. For use in this method, they suggest using any of a number of alternative aminopeptidase enzymes:

> The aminopeptidase enzyme used is preferably stable at a temperature up to about 65 °C, and stable and active at neutral pH, i.e. about 7.0, and at an alkaline pH, *i.e.* from about pH 8.0 to about pH 10.0. The aminopeptidase is preferably of a molecular weight of less than about 100,000, and of bacterial origin. The enzyme can be an extracellular aminopeptidase. In specific embodiments an aminopeptidase which is insoluble in water may be used. The aminopeptidase may also be used while it is bound to a solid support, or may be removed at the end of the reaction by use of an affinity resin.
>
> In a preferred embodiment of the invention the aminopeptidase is Aeromonas aminopeptidase. ***Other aminopeptidases may also be used***, such as Streptomyces griseus aminopeptidase and Bacillus stearothermophilus aminopeptidase II or III.[1]

All aminopeptidases, however, do not have the same activity.[2] When Blumberg et al. incubated a dissolved mixture of Aeromonas aminopeptidase and Cu_2-Zn_2 Human Superoxide Dismutase which has an N-terminal alanine, they found that it removed only 0.53 nmoles of the N-terminal alanine of 10.17 nmoles of the N-terminal residue in the sample. The amount of the removal was so trivial that they characterized it as a **non-removal**.[3]

Elsewhere in their patent, Blumberg et al. proposed that an aminopeptidase may remove an alanine from the N-terminal region of the alanine form of bGH:

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[1] Blumberg et al., U.S. Patent No. 5,763,215 at column 2, line 55 - column 3, line 3, emphasis added.

[2] See, *e.g.*, Blumberg et al., U.S. Patent No. 5,736,215 at column 1, line 38 - column 2, line 39; Kitazono, et al., J. Biochem. 116: 818-25 (1994) (disclosing an *Aeromonas sobria* aminopeptidase specific for amino-terminal proline).

[3] Blumberg et al., U.S. Patent No. 5,763,215 at column 16, lines 5-50.

> In specific embodiments of the invention the eucaryotic polypeptides are analogs of bovine growth hormone (bGH). These analogs contain the sequences Met-Asp-Gln or Met-Phe as their N-terminal sequence. The methionine is added to the N-terminus of these growth hormones when they are produced by recombinant DNA methods in bacteria. After removal of the N-terminal methionine by aminopeptidase, Asp-Gln-bGH and bGH are recovered respectively. The bGH used in this experiment was the phenyalanine form of bGH which has a phenyalanine residue as its N-terminus in its natural state. These methods also apply, however, to removal of N-terminal methionine from the terminus of the alanine form of bGH, which contains an alanine on the N-terminus of its natural form although in this case the alanine residue ***may*** also be removed.[4]

Significantly, however, Blumberg et al. did not state that the alanine ***would*** be removed, only that it ***may*** also be removed. In addition, Blumberg et al. failed to disclose which of the number of aminopeptidases they propose ***may possibly*** remove this alanine. In view of Blumberg et al.'s characterization of the action of Aeromonas aminopeptidase upon the N-terminal alanine residue of Cu_2-Zn_2 Human Superoxide Dismutase **as a non-removal**, a person of ordinary skill could only logically conclude that to the extent an aminopeptidase could possibly remove an N-terminal or N-terminal region alanine from a polypeptide it must be an aminopeptidase **other than** Aeromonas aminopeptidase.

Against this backdrop, the patentability of the subject matter of claim 22 must be examined. Claim 22 requires **expressing** a peptide having an alanyl residue in the N-terminal region and **contacting** the expressed polypeptide with **immobilized** *Aeromonas proteolytica* aminopeptidase to cleave the alanyl residue. Blumberg et al. do not suggest such a method. If anything, they would have led a person of ordinary skill away from this method: Blumberg et al. characterized their sole attempt to remove an N-terminal alanine from a protein in a dissolved mixture of the protein and Aeromonas aminopeptidase as a non-removal. To arrive at the invention defined by claim 22, therefore, a person of ordinary skill would have needed to disregard Blumberg et al.'s sole example (when there is no apparent reason for doing so) and nevertheless express a peptide having an alanyl residue in the N-terminal region of the

[4]Blumberg et al., U.S. Patent No. 5,763,215 at column 5, lines 43-57, emphasis added.

polypeptide and contact the expressed polypeptide with **immobilized** *Aeromonas proteolytica* aminopeptidase.

Pedersen et al. disclose nothing to bolster the expectation of success of the use of Aeromonas aminopeptidase to remove an alanyl residue from the N-terminal region of a polypeptide. Specifically, Pedersen et al. disclose the use of aminopeptidases to remove N-terminal residues or combinations of residues. Pedersen et al. passingly note that Aeromonas aminopeptidase, among others aminopeptidases, could also be used to achieve good results. While the Office cites Example 9 of Pedersen et al. as disclosing the use of the combination of dipeptidyl aminopeptidase I (DAP I), Aeromonas aminopeptidase (AAP), and glutamine cyclotransferase (GCT) to remove a series of 12 amino acids, including an alanine, from StrepTag1-TNFα in order to generate authentic TNFα, it appears that the Office's interpretation is incorrect. Although Example 9 states the phrase "Biotinylated AAP" (Biotinylated Aeromonas aminopeptidase), it is clear from the context of Example 9 and the Methods and Materials section of the specification, taken together, that the phrase "Biotinylated AAP" is a typographical error, and that the use of Biotin-**APP** (Biotinylated aminopeptidase P) to remove an amino acid tag is what is actually disclosed in the example.[5]

Specifically, Example 9 correctly discloses the phrase "Biotin-APP" twice within this example – once with respect to the use of APP to cleave portions of the 12 amino acid tag,[6] and once with respect to the removal of APP from the cleaved sample.[7] Moreover, the self-contained Experimental Details section of Pedersen et al. contains a Materials and Methods section[8] that describes the preparation of **all** enzymes used in the Examples, including Example 9. This Methods and Materials section describes **only** the preparation of DAP I, GCT, PGAP, and APP. Noticeably, there is **no** description of the preparation of AAP. These points taken together, one

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[5] *See*, Pedersen et al., Example 9, Column 12, line 1 et seq., and in particular, lines 22-28.

[6] Pedersen et al., Column 12, lines 20-24.

[7] Pedersen et al,., Column 12, lines 24-29.

[8] Pedersen et al., Column 6, lines 15-50.

would be left to conclude that the parenthetical phrase " . . .(Biotinylated-AAP . . .) . . ." in Example 9 is a typographical error (Biotinylated-APP being intended), and that Biotin-APP, rather than Biotin-AAP, was the aminopeptidase used in Example 9. Accordingly, Pedersen et al. do not disclose the use of Aeromonas aminopeptidase to remove an N-terminal alanyl group from a polypeptide.

Likewise, Obata et al. (JP 07289256) and Obata et al. (1997) also do nothing to aid one in achieving the invention of claim 22. These two references merely disclose the characterization of a novel aminopeptidase, aminopeptidase K, isolated from *Aeromonas salmonicida*, and having a substrate specificity in which the highest rate of hydrolysis is observed with a substrate having alanine as the N-terminal residue.[9] While the Office asserts that it is well known in the art of enzymology that closely related organisms produce similar enzymes with similar activities and similar specificities, it is also well known in the art that organisms within even the same genus produce enzymes that share neither similar activities nor similar specificities. Specifically, *Aeromonas sobria* produces an aminopeptidase that demonstrates almost absolute specificity for amino-terminal proline.[10] Therefore, the simple disclosure of the activity and specificity of a single aminopeptidase from a single *Aeromonas* species is not necessarily indicative of the activities and specificities of all other aminopeptidases from all other *Aeromonas* species, nor would a person of skill in the art expect one such aminopeptidase to necessarily be indicative of another.

Although Harper et al. disclose the use of Aeromonas aminopeptidase, they do not disclose the use of the same to remove an alanyl residue from a polypeptide, but instead only to remove a methionine residue.

These references, when considered in combination with Blumberg et al., simply would not motivate anyone to arrive at the claimed invention nor would it have allowed one skilled in the art to anticipate with any degree of certainty the now-demonstrated utility and success of the

[9] See Obata et al. (1997) at p. 1107, right column, third full paragraph.

[10] Kitazono, et al., J. Biochem. 116: 818-25 (1994).

method of claim 22. Absent all of this, the Office has failed to establish a *prima facie* case of obviousness as to claim 22.[11]

Claims 23-34, which depend from claim 22, are patentable over Blumberg et al., and Pedersen et al. in view of Harper et al. and Obata et al. (JP 07289256) or Obata et al. (1997) for all reasons stated above with respect to claim 22 and by reason of the additional requirements which they introduce.

Claim 35 is directed to a method of removing an N-terminal alanyl residue from a polypeptide. The method comprises expressing a polypeptide having an N-terminal alanyl residue and contacting the expressed polypeptide with immobilized *Aeromonas proteolytica* aminopeptidase to cleave the N-terminal alanyl residue from the polypeptide. Blumberg et al., Pedersen et al., Harper et al., Obata et al. (JP 07289256) and Obata et al. (1997) do not individually, or in combination, suggest expressing a polypeptide having an N-terminal alanyl residue and then cleaving it with immobilized *Aeromonas proteolytica* aminopeptidase. If anything, Blumberg et al.'s Example 10 would suggest this is something to be avoided.

Claims 36-47 depend from claim 35 and are patentable over Blumberg et al., Pedersen et al., Harper et al., Obata et al. (JP 07289256) and Obata et al. (1997) for all reasons stated above with respect to claim 35 and by reason of the additional requirements which they introduce.

Claim 48 is directed to a method of removing an N-terminal alanyl residue from a recombinantly expressed polypeptide having an N-terminal alanyl residue. The method comprises contacting the expressed polypeptide with immobilized *Aeromonas proteolytica* aminopeptidase to cleave the N-terminal alanyl residue from the polypeptide. Blumberg et al., Pedersen et al., Harper et al., Obata et al. (JP 07289256) and Obata et al. (1997) do not individually, or in combination, suggest contacting a recombinant polypeptide having an N-terminal alanyl residue with immobilized *Aeromonas proteolytica* aminopeptidase to cleave this residue. If anything, Blumberg et al.'s Example 10 would suggest this is something to be avoided.

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[11] MPEP §2142.

Claims 49-60 depend from claim 48 and are patentable over Blumberg et al., Pedersen et al., Harper et al., Obata et al. (JP 07289256) and Obata et al. (1997) for all reasons stated above with respect to claim 48 and by reason of the additional requirements which they introduce.

Claim 61 is directed to a method of removing an alanyl residue from the N-terminal region of a polypeptide. The method comprises expressing a polypeptide having an alanyl residue in the N-terminal region and contacting the polypeptide with mobilized *Aeromonas proteolytica* aminopeptidase to cleave the alanyl residue from the polypeptide. Blumberg et al., Pedersen et al., Harper et al., Obata et al. (JP 07289256) and Obata et al. (1997) do not individually, or in combination, suggest contacting a polypeptide having an alanyl residue in the N-terminal region with mobilized *Aeromonas proteolytica* aminopeptidase to cleave this residue. If anything, Blumberg et al.'s Example 10 would suggest this is something to be avoided.

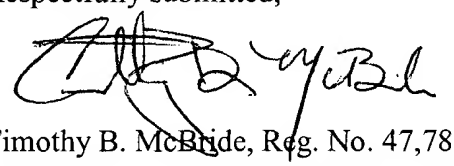
Claims 61-68 depend from claim 60 and are patentable over Blumberg et al., Pedersen et al., Harper et al., Obata et al. (JP 07289256) and Obata et al. (1997) for all reasons stated above with respect to claim 60 and by reason of the additional requirements which they introduce.

CONCLUSION

In light of the above arguments, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. 103(a).

Applicants request an extension of time to and including April 7, 2003, for filing this amendment. The Commissioner is hereby authorized to charge any deficiency or overpayment in connection with this amendment to Deposit Account No. 19-1345.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

Claims 22-68 have been newly added.

Claims 1-3 and 5-21 have been cancelled.